MENDELIAN AND CYTOPLASMIC INHERITANCE IN YEASTS[1]

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The standard ellipsoidal cell of *Saccharomyces cerevisiae* is diploid (Kruis and Šatava, '18; Winge, '35). Under certain conditions its diploid nucleus undergoes meiosis and four haploid spores are produced (Lindegren and Lindegren, '44a; Lindegren and Hamilton, '44). One-, two-, three-, and four-spored asci are found, showing that many accidents may occur during the reduction division (Lindegren and Lindegren, '44a). In our work we select the four-spored asci and dissect out the four spores separately. Each ascospore grown alone produces a small cluster of round haploid cells. Genetical analysis has shown that the ascospores are of two kinds, *a* and *α* (Lindegren and Lindegren, '43b, '43c, '43d; Lindegren, '44). The legitimate diploid vegetative cells are formed by the fusion of *a* and *α* gametes, and these legitimate diploids produce four viable ascospores on reduction, thus completing the cycle. The haplophase cultures, when grown alone, often produce diploid cells by the copulation of two haplophase cells of the same mating type; we call these illegitimate (*a/a* and *α/α*) diploids because they only rarely produce four viable ascospores. The round-celled haplophase cultures often become stabilized in the haploid condition and gradually become incapable of mating with other forms after being carried in culture for some time (Lindegren and Lindegren, '44b). Asporogenous yeasts such as *Torulopsis* and *Asporomyces* probably originated in this manner.

SEGREGATION AND MUTATION

Haploid yeast cells are much smaller and more variable than diploid cells, varying more both from culture to culture and within a single culture than diploid cells. These differences are also reflected in the colonies, the diploid colonies being larger and more uniform, while haploid cultures produce smaller colonies which are usually rough and generally show considerable variation. The haplophase originates by the reduction of the diplophase at spore formation, and the

segregation of a heterozygote produces segregants of different genotypes. Yeasts are extraordinarily heterozygous, and a great variation of colonial forms is obtained by the isolation of single ascospore cultures. The haploid segregants are usually rough-colonied; smooth-colonied diploid cells usually produce only rough-colonied haploid segregants. Apparently considerable mutation occurs in the haplophase but generally the original segregant can be distinguished from the secondary mutants when the culture is plated out. At first, the mutants are usually slow-growing and produce small round colonies but on transfer they become adapted and stabilized and their specific colonial character becomes apparent, distinguishing them from the original segregant. Therefore, there are two mechanisms producing variation in yeasts explainable on purely genetical grounds (fig. 1):

(1) *Segregation.*—Segregation of genes of a heterozygous diploid at meiosis produces four spores, each of which develops a different type of colony.
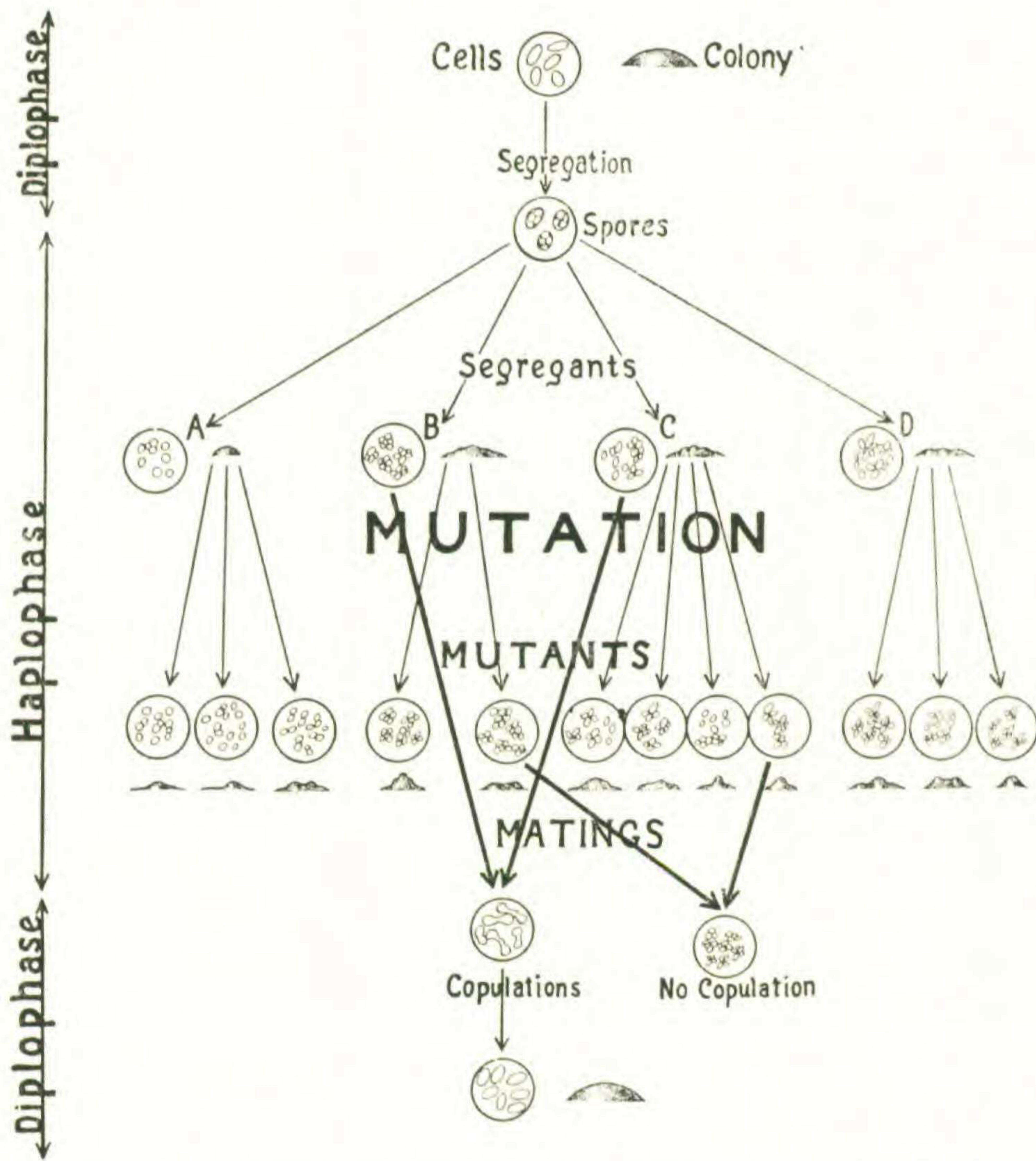

Fig. 1. Diagram showing the effects of segregation and mutation in producing variation in yeasts. The circles show the sizes and shapes of the cells as seen under the microscope and the stippled figures are profiles of the colonies on agar.

(2). *Mutation.*—Mutation in the haplophase enormously increases the variation of colonial forms. It is possible by continued subculturing to develop a tremendous variation of colonial forms from a single haplophase culture, but this usually results in loss of fertility.

DISTINCTION OF HAPLOPHASE AND DIPLOPHASE

Winge ('35) was the first to distinguish clearly between haplophase and diplophase yeast cultures, and we have corroborated his observations with some slight modifications. Workers familiar with other biological material may seriously question the propriety of speaking definitely of haplophase and diplophase in organisms where the cytological facts have not been conclusively demonstrated. I shall therefore summarize all the arguments, Winge's reinforced by ours, for distinguishing haplophase and diplophase. I should preface these rules by saying that over four-fifths of the cultures which one encounters are easily characterized by microscopic examination. They are either obviously haploid or diploid, as shown simply by size, shape, and aggregation of cells. The reasons for classifying them are as follows:

(1) The large vegetative cells which we call "legitimate diploids" produce viable four-spored asci. These spores germinate to produce smaller cells, which we call "haploid." The latter multiply vegetatively, generally maintaining their specific cell-shape and size.

(2) Two of these smaller cells may fuse to produce a large "diploid" cell capable of vegetative multiplication (Winge and Laustsen, '39a, '39b, Lindegren and Lindegren, '43b, '43d). While the large cell is undergoing vegetative reproduction, it retains its characteristic ellipsoidal shape and size. Under certain conditions, this diploid cell can be induced to sporulate. Spores from it in turn produce haploids and the process can be repeated indefinitely.

(3) The large cells which we recognize as diploids are extraordinarily stable in their genetical characteristics when they are grown under conditions in which sporulation does not occur. Transferring the cultures every forty-eight hours in broth is generally sufficient to maintain the vegetative diplophase. Colonies produced by plating out are not sectored; the plates do not show colonial variants. However, when haplophase (single ascospore) cultures of any age are plated out, a variety of colonial variants appear on the plate or the giant colonies are sectored. These facts are consistent with the view that the large cells are diploid, thus minimizing the number of spontaneous mutations found, while in the haplophase every mutant becomes apparent and is easily discovered.

(4) When the diploid cells sporulate to produce haploid cells, there is genetic evidence of a reduction division (Winge and Laustsen, '37). Genetical analysis shows that a single pair of alleles responsible for the two different mating types is segregated at this meiosis. Two *a* and two *α* type haplophase cultures are usually obtained from the four single ascospore cultures (Lindegren and Lindegren, '43d). There is also genetic evidence for the segregation of a gene-pair

controlling fermentation of melibiose (Lindegren, Spiegelman, and Lindegren, '44) during the meiosis that precedes spore formation. Also, evidence proving that factors controlling cell shape may be segregated in a hybrid of S. *Bayanus* and S. *cerevisiae* has been accumulated in addition to that previously offered by Winge and Laustsen ('39c) in the balanced heterozygote, *Saccharomycodes Ludwigii*.

(5) Haploid cultures of *a* and α mating type have been paired, and the resulting diploid cultures in turn have been induced to sporulate; the haplophases have been tested and found again to fall into the *a* and α categories. Matings and tests for this character have been carried through four or five generations in several cases. Similarly, segregation of the gene-pair controlling melibiose fermentation has been observed; the segregants have been tested, mated, and segregation has again been observed in the succeeding generation. Pedigrees of three or four generations are available for many characters.

The above facts seem to prove conclusively that the terms haplophase and diplophase can be used as definitely in speaking of yeasts as of organisms in which the cytological evidence is more complete. The illegitimate diploids provide an exception which is quite familiar to the mycologist. Copulations between haplophases which are usually incapable of copulating on genetical grounds were called "Durchbrechungskopulationen" by Brunswik ('24). Copulations of this type frequently occur in single ascospore cultures and produce diploids which are homozygous for the *a* or α factors. With rare exceptions these diploids sporulate poorly, and for this reason we have not studied them intensively. A few single ascospore cultures sporulate well, and some produce large cells that are difficult to classify either as definite haplophases or illegitimate diploids. However, the general rules laid down in the preceding discussion hold very well, and exceptions are not more frequent than one would expect on the basis of mutations, polyploidy, apomixis, or other genetical aberrations.

MATING TYPES

The variability of colonial characters is not paralleled by similar variation of mating-type specificity. With rare exceptions, each haplophase culture belongs to either the *a* or α mating type, or is sterile. A considerable number of sterile cultures are found and fertile cultures may become sterile, especially if the haplophase is carried a long time in culture. However, the mating types are differentiated primarily by a single pair of alleles.

A large-scale experiment has indicated that only two principal mating-type alleles are present in S. *cerevisiae*. Figure 2 shows the results of mating 58 different single ascospore cultures derived from a variety of industrial bakers' yeasts. Ly, HD, M, FLD, D, and B represent standard legitimate diploid strains of commercial baking yeasts; 800 is one of the baking strains of yeast obtained by Dr. Wickerham of the Northern Regional Research Laboratory, Peoria, Illinois. Two other cultures are hybrids, one of 800 x L and the other (800 x L) x L. Haplophases isolated from the "(800 x L) x L" hybrid were generally quite fertile.

Fig. 2. Results of matings between 58 haplophase single ascospore cultures in all combinations. The + sign indicates the occurrence of copulations; the — sign, that no copulations were found. The results indicate that the a/α alleles are generally distributed through a variety of strains of S. cerevisiae.

Three belong to mating type *a* and seven belong to mating type α. Two were sterile. Copulations invariably occurred when an *a* and α culture were mated, and haplophase 1A produced illegitimate matings with three other α type cultures. The Ly strain was also generally quite fertile. Three α type haplophases and seven *a* type haplophases were found in this culture. Only once did an *a* x α mating fail to produce fusions. When the "(800 x L) x L" haplophases were mated with the L haplophases, a high degree of fertility was demonstrated, with only seven failures out of forty-eight tests. With this strain also, culture 1A produced illegitimate diploids. This highly fertile culture was also able to mate with 2C, 3A, and 4B, which were incapable of producing fusions with any other culture with which they were tested. When the *a*-haplophases of the L and the "(800 x L) x L" hybrid were outcrossed to the other strains of yeast, only three hybrids were produced in several hundred matings, but outcrossing with the α strains was much more successful and resulted in a large number of hybrids. This occurred in spite of the fact that the HD, M, FLD, D, and B cultures were apparently quite in-fertile among themselves. It appears, therefore, that the α strains from this line can be successfully outcrossed to produce hybrids with other strains.

These results demonstrate that the *a*/α alleles obey the standard rules of Mendelian inheritance, and that other genes may apparently act as modifiers of mating type, generally resulting in reduced fertility.

CYTOPLASMIC ADAPTATION OF AN ILLEGITIMATE HYBRID

The illegitimate diploids are genetically stable forms because they sporulate rarely and, if transferred frequently in broth, will not sporulate at all. The failure to sporulate eliminates segregation as a cause of variation. Furthermore, diploids are practically free from spontaneous mutations because each locus is "covered" by a dominant normal allele. We have studied adaptation to a specific environment, using an illegitimate diploid. Adaptation to a carbohydrate-peptone mash which contained an unknown substance that inhibited yeasts was studied. The first transfer made from malt medium to this carbohydrate-peptone mash grew very poorly, but adaptation to the new medium always occurred on the second serial transfer.

The malt medium (M) contained 10 per cent malt extract, 0.5 per cent dextrose, 0.5 per cent dried yeast, 1 per cent $CaCO_3$, 3 per cent agar. The carbo-hydrate-peptone mash medium (C) contained 0.8 per cent sucrose, 0.7 per cent nitrogen-containing solids, 1 per cent $CaCO_3$, 3 per cent agar. M agar is a rela-tively complete medium which supports an abundant growth of uniformly large colonies. Adaptations of different types of yeast to these media have been reported (Lindegren and Lindegren, '43b). On the first transfer to C agar only a small percentage of cells survives, and the variations in colony-size on this agar are not due to genetic differences. Figure 3 shows the results of plating serially on M and C media.

One of the large colonies from an M plate was suspended in water, and equal
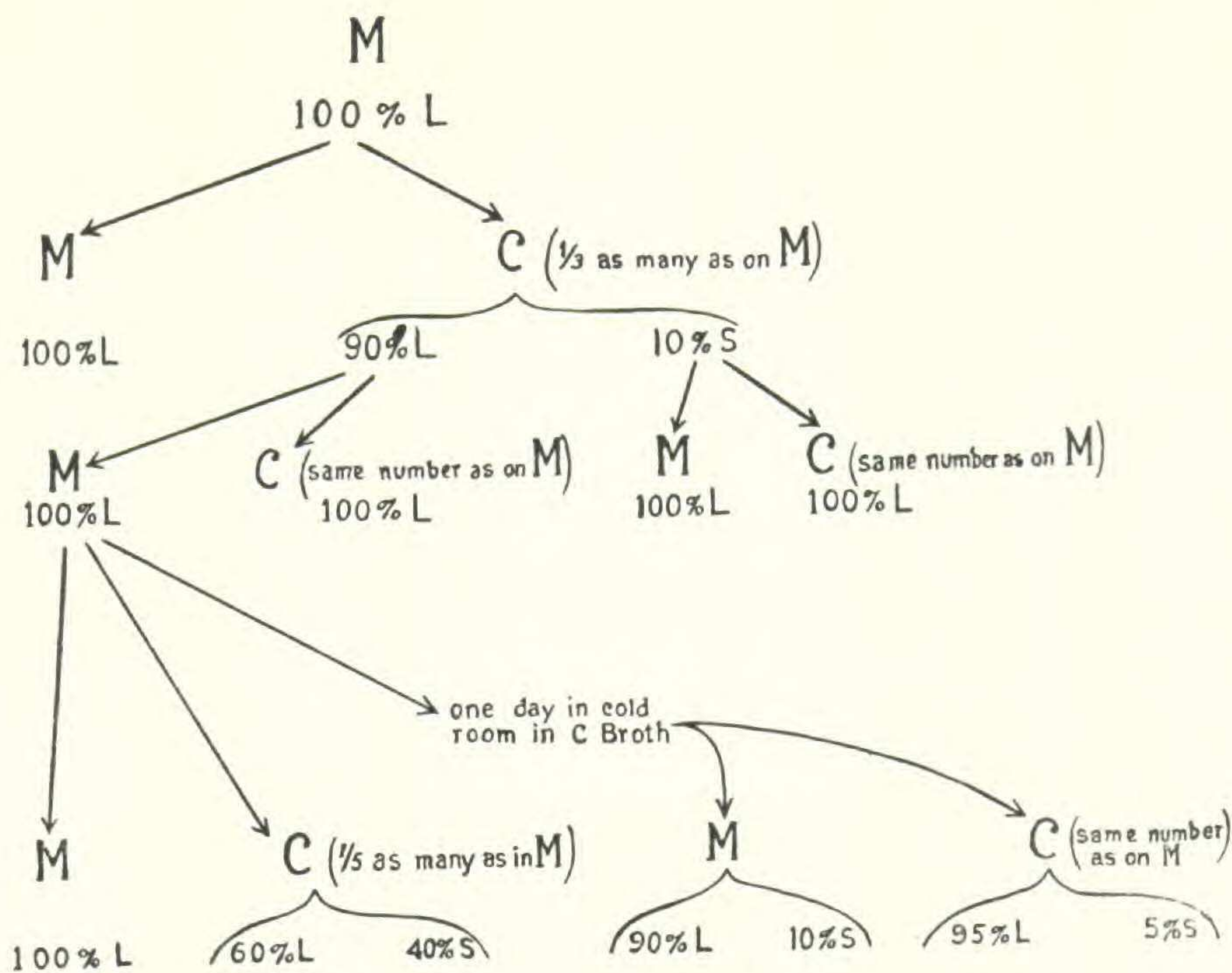
Fig. 3. Diagram showing adaptation of an illegitimate diploid (in which mutations are only rarely expressed) to an unfavorable medium.

amounts of the suspension planted on M and C plates. All the colonies appearing on the M plate were uniformly large. Only about one-third as many colonies appeared on the C plate, and of these 90 per cent were large and 10 per cent were small. In all transfers a large number of cells die; from 10 to 30 per cent of the cells transferred from M to M usually produced colonies, but the mortality is always greater on the first transfer from M to C than on transfers from M to M. Since the genotype is stabilized any selection must be for some difference, independent of the genotype.

A suspension was made of a large colony growing on a C plate, and equal amounts of the suspension plated on M and C plates. The same number of colonies appeared on both plates and all were full size. This proved that the survivors on the C plate were adapted to growth on C agar. A suspension of a small colony gave the same result. The fact that there is no detectable difference between the large and small colonies on C plates indicates that the small colonies are merely slower in development. If a colony is delayed in development, the staling effect of the more rapidly developing colonies on the medium will prevent it from attaining full normal size.

Equal amounts of a suspension from one of the large colonies on an M plate (descended from a colony on a C plate) were plated on M and C plates. Only one-fifth as many colonies appeared on the C plate as on the M plate and both large and small colonies were found. Therefore, cells growing on a C plate (which have become adapted to C agar) lose this adaptation by a single transfer to M agar. This confirms the fact that the first transfer to a C plate did not select genotypes.

It was also possible to adapt the cells to C agar by holding them in C broth in the cold room for two days. A large colony from an M plate was suspended in C broth and held two days in a cold room. When samples from this culture were spread on M and C plates a small number of colonies appeared on both media, although the samples of the untreated culture plated directly from M plates to both M and C plates showed only one-fifth as many colonies on the C as on the M plate.

Since the C medium is obviously unfavorable to the cells coming directly from the M medium it seems probable that the former contains some harmful substance. However, interaction between the cytoplasm and the C medium results in an adaptation apparently enabling the illegitimate diploid to produce a metabolite capable of neutralizing this substance, and this metabolite continues to be produced as long as contact with C medium is maintained. This adaptation must be cytoplasmic because no change has occurred in the genotype. The metabolite which neutralizes the C substance may have been absent from the yeast cells or may be merely increased in amount during adaptation to the C medium. This type of non-genic variation constitutes a complication in the analysis of yeast genetics, and experiments must be designed so that it can be distinguished from the variations resulting from segregation and mutation.

Winge and Laustsen ('40) have demonstrated that a cytoplasmic deficiency may occur in yeasts when a nuclear division is not accompanied simultaneously by a cell division. They have assumed that this condition results from a deficiency of chondriosomes. Their phenomenon is apparently quite different from adaptation of the cell to C medium in which an interaction of substrate and cytoplasm is involved.

MENDELIAN INHERITANCE OF AN ADAPTIVE ENZYME

S. cerevisiae is incapable of fermenting melibiose, and its haploid segregants fail to ferment this sugar even after continued growth in broth containing melibiose. This indicates that mutations enabling the yeasts to ferment the sugar either do not occur in this species or else that they are extremely rare. *S. carlsbergensis* is capable of fermenting melibiose, as are all its haploid segregants. This is the principal character upon which *S. cerevisiae* and *S. carlsbergensis* are differentiated. Figure 4 is a pedigree describing the progenies of matings between these two species (Lindegren, Spiegelman and Lindegren, '44). The data were obtained by growing the cultures in a broth tube containing a smaller inverted tube to collect the gas produced by fermentation. Accumulation of gas in the inverted tube is indicated by a plus sign.

Hybrid I was an interspecific hybrid (*cerevisiae* x *carlsbergensis*) made by mixing melibiose-plus and melibiose-minus haplophase cultures. Three diploid cells isolated after this mating were all capable of fermenting melibiose. Eight asci were dissected from interspecific hybrids, and all the haplophase progeny were tested for the ability to ferment melibiose. The results showed that the haplo-
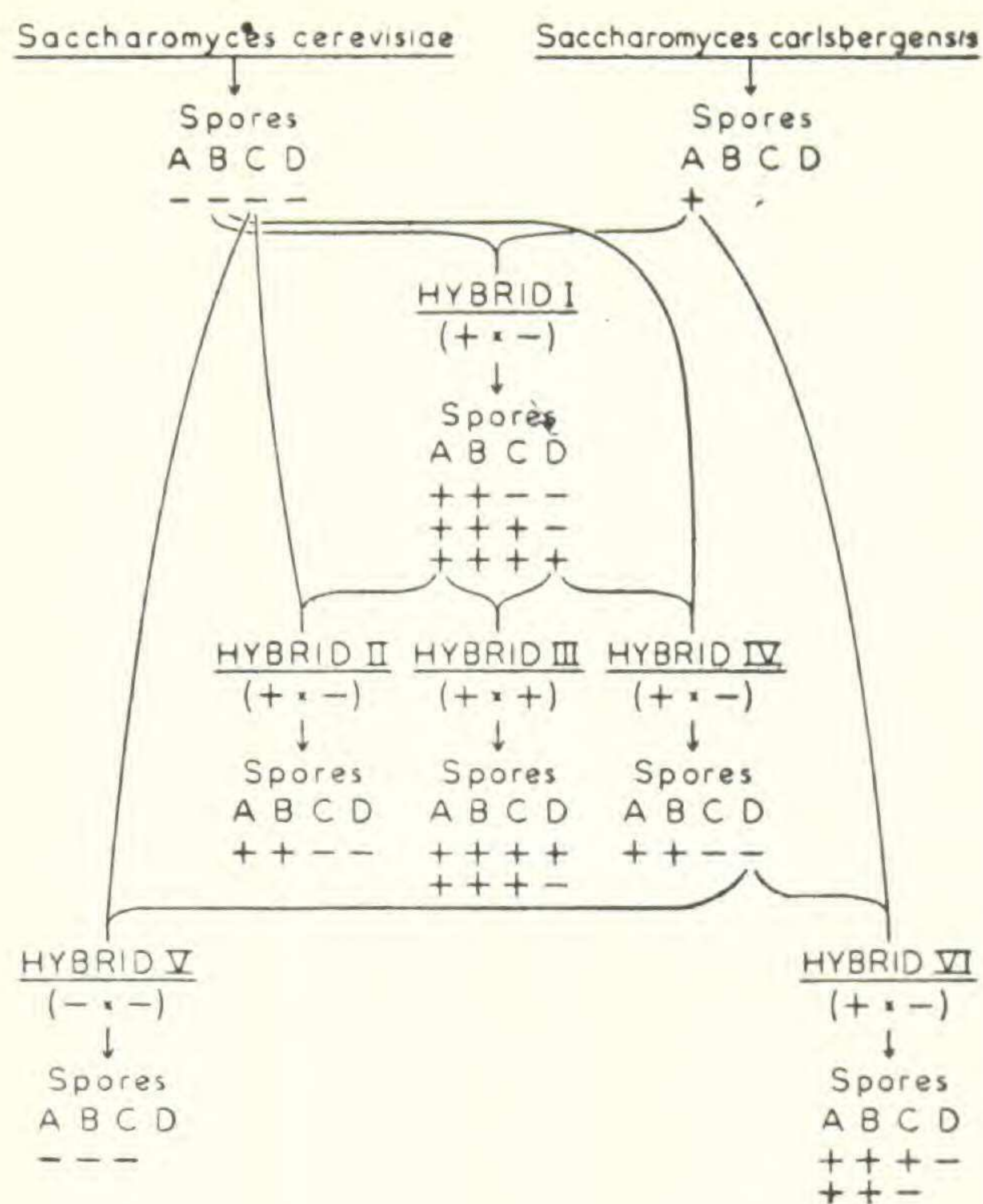
Saccharomyces cerevisiae   Saccharomyces carlsbergensis
    ↓   ↓
  Spores   Spores
  A B C D   A B C D
  – – – –   +

            HYBRID I
             (+ • –)
               ↓
             Spores
             A B C D
             + + – –
             + + + –
             + + + +

HYBRID II   HYBRID III   HYBRID IV
 (+ • –)   (+ • +)   (+ • –)
    ↓   ↓   ↓
 Spores   Spores   Spores
 A B C D   A B C D   A B C D
 + + – –   + + + +   + + – –
              + + + –

HYBRID V   HYBRID VI
 (– • –)   (+ • –)
    ↓   ↓
 Spores   Spores
 A B C D   A B C D
 – – –   + + + –
                                  + + –

Fig. 4. Fermentation of melibiose by diploid and
haploid progeny of *Saccharomyces cerevisiae* x *S. carls-
bergensis* hybrids; + sign signifies fermentation of meli-
biose, — sign, inability to ferment melibiose.

phase cultures from three asci were melibiose +; two asci produced three + and
one — culture; and one ascus produced two + and two — cultures. These ratios
indicate that more than one gene is involved.

Hybrid II was produced by backcrossing a positive haplophase culture from an
ascus producing four + cultures with a negative haplophase culture from *S.
cerevisiae*. A regular Mendelian segregation of the progeny shows that the haplo-
phase carried a single gene capable of controlling melibiose fermentation.

Hybrid IV was made by backcrossing a second positive haplophase culture
from the same ascus to a negative haplophase culture from *S. cerevisiae*. In this
case, a regular Mendelian segregation again shows it also carried a single gene.

Hybrid III was produced by mating the two positive cultures, each of which
carried a single gene. Analysis of hybrids II and IV proved that each of these
cultures carried a single gene controlling melibiose adaptation. If these genes
were alleles, all the haplophase progeny of the hybrid should ferment melibiose.
Since two of the twenty haplophase segregants failed to ferment melibiose, the
original culture of *S. carlsbergensis* must have contained two different non-allelic
loci controlling melibiose fermentation.

Hybrid V was made by backcrossing a negative haplophase segregating from
the hybrid to a negative haplophase from *S. cerevisiae*. The three haplophase
progeny were all negative.

Hybrid VI was made by backcrossing the same negative culture to a positive haplophase of S. *carlsbergensis*. Five of seven haplophase progeny fermented melibiose, while two failed. This finding, together with the results obtained in hybrid V, confirms the fact that the original haplophase culture of S. *carlsbergensis* possessed two genes controlling melibiose fermentation.

This pedigree is of especial interest because Dr. Spiegelman was able to show that the fermentation of melibiose is under the control of an adaptive enzyme (Kärstrom, '38). Twelve critical cultures were tested in the Warburg apparatus to determine whether fermentation occurred immediately or whether adaptation to the substrate was required, i. e., whether fermentation only occurred after a period of exposure to melibiose. In each case it was found that an adaptive enzyme was involved. The adaptation time was not the same for each strain, which agrees with previous work (Spiegelman and Lindegren, '44), but the time for each is specific and is reproducible under standard conditions.

In the inverted-tube method, part of a clone is seeded into the broth containing melibiose as the carbohydrate source and allowed to grow. Since every mutation in a haplophase population becomes functional immediately, a positive test might not mean that the original clone possessed the fermentative capacity. Selection of a mutant produced during growth in the melibiose solution may have occurred (Spiegelman, Lindegren and Hedgecock, '44). The Warburg tests of the twelve critical cultures excluded this possibility by the fact that in the Warburg apparatus adaptation occurred in a stationary population. If a stationary population exposed to melibiose acquires the ability to ferment the sugar, it can only be due to an interaction between the existing cells and the sugar. Cells which have been adapted to ferment melibiose lose this ability when removed from the substrate and have to be readapted to use it fermentatively.

MAINTENANCE AND INCREASE OF MELIBIOZYMASE IN THE ABSENCE OF THE SPECIFIC GENE

In the preceding experiments on adaptation to melibiose the first contact with the substrate occurred when the culture was transferred to a fermentation tube containing melibiose. A second series of experiments (Spiegelman, Lindegren, and Lindegren, '45) showed that if contact with melibiose were maintained during the growth of the haplophase cultures, during copulation, during growth on the presporulation agar, and during spore formation, all the segregants from heterozygous diploids, such as hybrids II and IV, carrying a single pair of genes, were able to adapt to melibiose fermentation. However, two of the melibiose-plus cultures from each ascus completely lost their ability to ferment melibiose when vigorously dissimilated. This proves that melibiozymase was transferred from the cytoplasm of the heterozygous hybrid which had been maintained on melibiose to the cytoplasm of the haplophase segregants which did not carry the specific gene. Furthermore, the melibiozymase was maintained in the segregants which carried the melibiose-plus gene by an interaction between melibiozymase and melibiose. There-
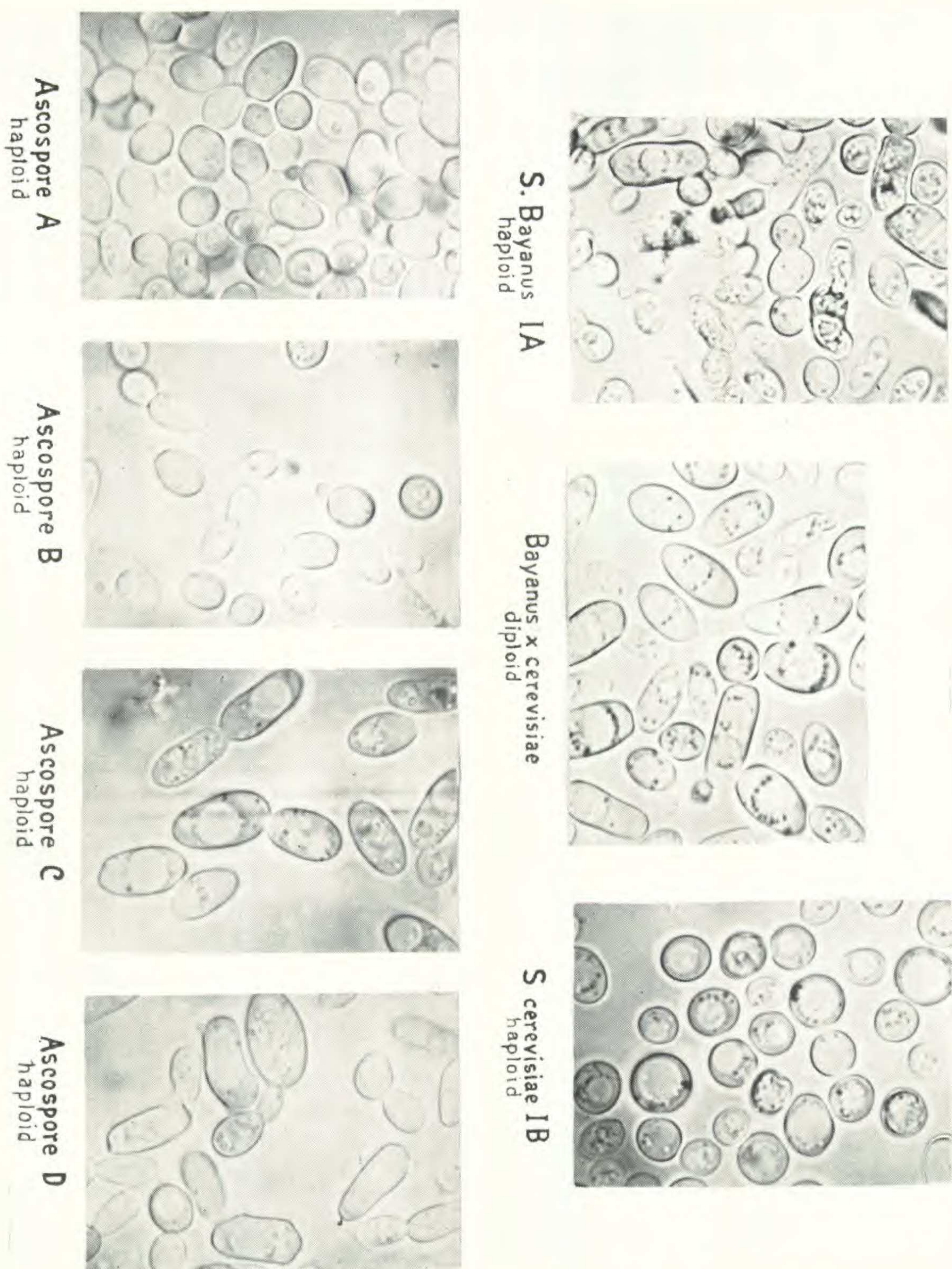
Fig. 5. Photographs of the haplophase cells of *Saccharomyces Bayanus* and *S. cerevisiae* and the diplophase cells of the interspecific hybrid. A, B, C, and D are the haploid cells grown from the four spores of a single ascus of the hybrid.

fore, melibiozymase is a self-perpetuating cytoplasmic entity which is gene-initiated, but the quantitative level of melibiozymase below the maximum depends on an interaction between melibiose and the enzyme and is independent of the gene.

Since S. *carlsbergensis* is homozygous for two pairs of genes which produce melibiozymase, there are four loci in the diplophase of this organism capable of producing this enzyme. There are four corresponding recessive alleles in S. *cerevisiae*, which is probably the most cosmopolitan and best established yeast species. It seems improbable that a successful wild type yeast should carry four functionless genes.

INHERITANCE OF ADAPTATION TO GALACTOSE

The fermentation of galactose by S. *cerevisiae* is due to an adaptive enzyme similar to that produced by S. *carlsbergensis* for the fermentation of melibiose. Since S. *Bayanus* is incapable of fermenting galactose, hybrids between it and S. *cerevisiae* make it possible to study the inheritance of galactose adaptation. There is one advantage in this particular case, namely, that S. *Bayanus* produces large cylindrical cells both in the haplophase and diplophase, providing an additional genetical marker. The hybrid between the large cylindrical (L) gametes of S. *Bayanus* and the round (l) gametes of S. *cerevisiae* produced a large cylindrical (L) diplophase, proving that the *Bayanus*-type cell is dominant. One difficulty is that our culture of S. *Bayanus* sporulated only rarely and only one ascospore of a very large number that was isolated grew. The fact that many of the single ascospore cultures of the hybrid produced viable four-spored asci considerably complicated the genetical analysis. It is notable as an evidence of hybrid vigor that the original hybrid and the progeny all sporulated very abundantly in spite of the poor sporulation of the original S. *Bayanus*.

Figure 6 is a pedigree showing the progenies of a hybrid between S. *Bayanus* and S. *cerevisiae*. All of the haplophase cultures from hybrid IV fermented galactose (+). Half of the single ascospore cultures had large cylindrical (L) cells like S. *Bayanus* and half resembled haplophases of S. *cerevisiae* (l). The large cylindrical *Bayanus*-type cultures fermented galactose more slowly (L + slow) when studied by the inverted-tube technique than did the *cerevisiae*-type (l + fast) cells. The slow fermentation of some of the cultures growing in the fermentation tubes was probably due simply to the slower growth of the *Bayanus*-type segregants.

An analysis of some of the single-ascospore cultures which produced four-spored asci showed that the eight ascospores obtained from two asci isolated from diploid XIII (illegitimate IV-1A) were unable to ferment galactose. This proves that ascospore IV-1A did not carry the gene controlling galactose fermentation but was able to ferment galactose because galactozymase had been carried over cytoplasmically, just as melibiozymase had been carried over in the previous experiments. Our earlier experiments had shown that galactozymase, like melibiozy-

I
S. Bayanus

	A	B	C	D
1	L-	d	d	d

II
S. cerevisiae

	A	B	C	D
1	l+	l+	d	d

IV
I IA x II IB (diploids L+)

	A	B	C	D
1	L+slow	L+slow	l+fast	l+fast
2	l+fast	l+fast	L+slow	L+slow
3	l+	l+	L+	L+
4	d	d	d	d
5	l+fast	L+slow	L+slow	l+fast

XIII
IV IA (illegitimate)

	A	B	C	D
1	-	-	-	-
2	L-	L-	L-	irreg-

XIV
IV 5C (illegitimate)

		A	B	C	D
1	colony	L+ sm.	L+ lge.	D+ lge.	L+ sm.
	Warburg a.	+	+	+	+
	Adaptation b.	-	-	+	-
2	colony	+ sm.	+ sm.	+ lge.	+ lge.
3	colony	cop. sm.	sm.	D lge.	cop. lge.

XII
I IA x IV 5C

		A	B	C	D
1		L+fast	L+slow	L+slow	L+fast
	Warburg a.	+	+	-	+
	Adaptation b.	+	+	-	-
Plated Colonies	Small {+	3	3	0	2
	{-	0	0	8	8
	Large {+	3	2	0	11
	{-	0	0	9	1
2		+	+	+	d
3		+	+slow	+slow	+fast
	Warburg a.	-	-	+	-
	Adaptation b.	-	-	-	+

XVI
XIV IB (illegitimate)

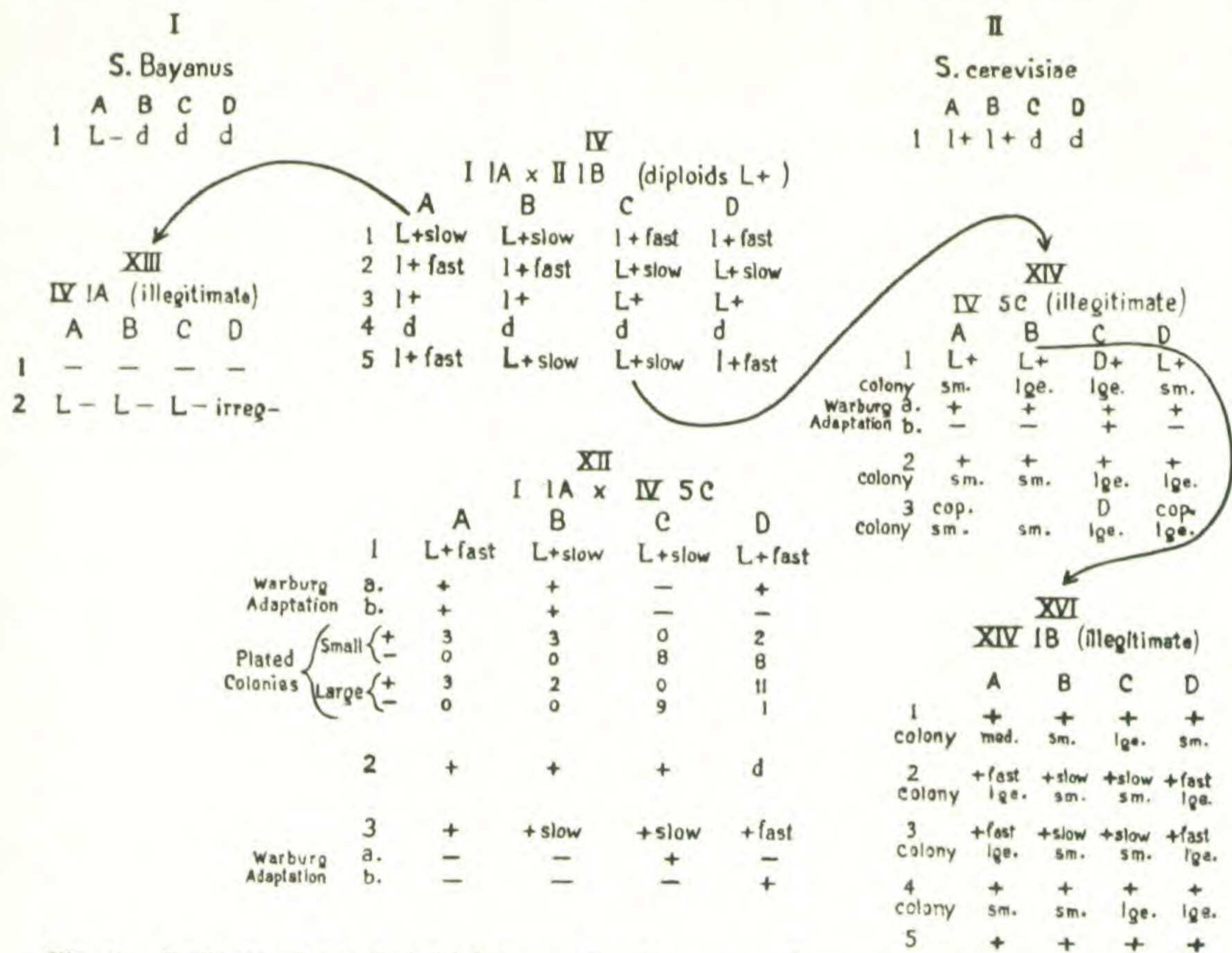|		A	B	C	D
1	colony	+ med.	+ sm.	+ lge.	+ sm.
2	colony	+fast lge.	+slow sm.	+slow sm.	+fast lge.
3	colony	+fast lge.	+slow sm.	+slow sm.	+fast lge.
4	colony	+ sm.	+ sm.	+ lge.	+ lge.
5		+	+	+	+

Fig. 6. Pedigree of the hybrid between *Saccharomyces Bayanus* and *S. cerevisiae* and the progeny from it. Discussed in text.

mase, is also substrate-dependent (Spiegelman, Lindegren, and Hedgecock, '44; Spiegelman and Lindegren, '44).

Diploid XIV (IV-5C illegitimate) was L+ and produced viable four-spored asci, and all the single ascospore isolates from it were capable of fermenting galactose. Warburg tests for adaptation to galactose revealed that although all four cultures from ascus 1 adapted within 5 hours on the first trial, three failed to adapt within this period on a second trial. Three asci were dissected from this illegitimate diploid, and each ascus yielded two small- and two large-colony cultures. This suggests that the illegitimate diploid was heterozygous and indicates that copulations in the single ascospore culture had occurred after mutations made the haplophase heterogeneous. Some of the segregants were poorly viable degenerate cells (D) and some produced an abundance of copulations (cop) in the haplophase cultures. These characteristics suggest a similarity to the cytoplasmic deficiencies found in illegitimates by Winge and Laustsen.

Diploid XVI (XIV-1B illegitimate) is the second inbred illegitimate generation derived from IV-5C. Two large- and two small-colony isolates were obtained from each ascus, indicating that the second generation is heterozygous like the first. All the isolates ferment galactose in the inverted-tube tests, and this was confirmed by a second test showing that the original culture, IV-5C, carried the + gene from *S. cerevisiae* together with the *Bayanus*-type cell.

Three asci were dissected from hybrid XII (an L segregant, IV-5C, carrying

the + gene, backcrossed to S. *Bayanus* I-1A, L—). All four ascospores from ascus 1 produced *Bayanus*-celled cultures capable of fermenting galactose. However, when they were grown on sucrose and then tested five hours in the Warburg for adaptation to galactose only A and B proved adaptable in both tests within this period. When all four haploid cultures were plated on agar and single colonies fished and tested for the ability to ferment galactose, A and B produced only fermenting cultures while most of the colonies fished from C and D failed to ferment galactose. These facts indicate that ascus 1 was heterozygous for the +/— alleles.

THE CYTOGENE HYPOTHESIS

These experiments on adaptation show that the ability of different yeasts to adapt themselves to specific substrates is due to a cytoplasmic mechanism. No genetical analysis was available in the case of the adaptation of the illegitimate diploid to C medium, but it was possible to show that genes control the adaptation to melibiose by making hybrids between S. *carlsbergensis* and S. *cerevisiae*, and similarly that genes control the ability to adapt to galactose by making hybrids between S. *cerevisiae* and S. *Bayanus*. In the latter experiments it was clear that although genes initiated the production of the adaptive enzymes, adaptation only occurred by interaction of the cytoplasm of the cells with the specific substrate; and furthermore, the adaptive enzyme, once it had been formed, was self-perpetuating in the presence of the substrate. This was further confirmed in both cases by showing that the adaptive enzyme could be transmitted through the cytoplasm and maintained in cells without the gene.

I propose to call adaptive enzymes of this type *cytogenes*. The fact that a period of exposure to melibiose must occur before the melibiozymase is produced

Nucleus   Cytoplasm   Environment

gene   protocytogene   substrate

M ————→ Z   melibiose
              cytogene
                  Mel+Z
        (self-perpetuating in presence
         of melibiose)

Fig. 7. Diagram explaining the cytogene hypothesis.

suggests that the original product is a relatively non-specific substance which is transformed into melibiozymase when it is "imprinted" by melibiose. The original relatively non-specific substance I propose to call the *protocytogene*. This concept is shown diagrammatically in fig. 7.

If the melibiose-plus gene produces a protocytogene which becomes a specific cytogene, melibiozymase, by being "imprinted" by the melibiose molecule, it is possible that the same locus may be responsible for the production of other cytogenes as well. The original gene-product which becomes specific by contact with the melibiose molecule might presumably become differently specific on contact with some other molecule. Genes are "enzyme factories," but each gene may not necessarily be restricted to the production of a single enzyme. The possibility that a single gene may produce a variety of cytogenes may be a different phenomenon from the one first described by Dobzhansky ('27) as the "manifold effects of a single gene."

DISCUSSION

Darlington ('44) has named certain self-perpetuating cytoplasmic entities which seem to be relatively independent of the genome *plasmagenes*. The cytogene differs fundamentally from the plasmagene, for the former, as defined above, is gene-initiated and substrate-dependent. However, a cytogene might possibly be transformed into a plasmagene by a metabolic mechanism which would synthesize the appropriate molecules within the cell. For example, if S. *cerevisiae* synthesized melibiose, melibiozymase could be maintained permanently in the hybrids as a constitutive enzyme. Such a mechanism might arise by mutation. Therefore, plasmagenes and cytogenes might be phylogenetically related in the following sequence: gene → cytogene → plasmagene.

Darlington has suggested that plasmagenes may evolve into viruses by mutation; however, this implies that plasmagenes are relatively independent entities more or less at the gene level. The preceding discussion suggests that most plasmagenes may be highly dependent on internal substrate for perpetuation rather than relatively independent as Darlington has suggested. The fact that the plasmagenes reproduce exclusively in a specific cytoplasm may mean that their actual existence depends upon contact with some specific type of molecule peculiar to that specific cytoplasm rather than on general "good" growing conditions in the cytoplasm. However, viruses seem to be relatively independent on the substrate and to resemble genes much more than either plasmagenes or cytogenes. Viruses might arise directly from genes rather than from plasmagenes. I ('38) have presented an hypothesis suggesting that viruses may evolve from genes by passage through an insect vector which may have some advantages over the hypothesis suggested by Darlington.

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BIOCHEMICAL GENETICS OF NEUROSPORA

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The production by irradiation of mutant strains of *Neurospora* (Beadle and Tatum, '41) has provided material for the cooperative attack by genetical and biochemical methods on the problem of the mechanism of gene action (Tatum, '44, Horowitz *et al.*, '45, Beadle, '45a). The genetic approach has shown that of the mutant strains so far investigated, both the morphological and biochemical ones are differentiated from normal by single genes. Genetic methods based on crosses and on the formation of heterocaryons have been developed for establishing the allelic or non-allelic nature of specific genes, and for determining the relative dominance of particular biochemical genes (Beadle and Coonradt, '44). The genetic analysis of mutant strains should in time provide data for the location of the mutant genes on chromosome maps. Already quite a few genes have been so located on one or another of the seven chromosomes of *Neurospora* recently demonstrated by McClintock (unpublished).

The biochemical investigations have supported the view that genes controlling biosyntheses of vitamins and amino acids and other biologically important substances in *Neurospora* act through their primary effect in determining the specificity or the production of enzymes involved in carrying on individual steps in the biosyntheses. The mutant strain is perhaps characterized by total or partial failure of enzyme synthesis. Another possibility would be the production of a modified enzyme with altered specificities, which is as a result either inactive or less effective than the normal enzyme in catalyzing the required reaction. If the general concept of a biosynthesis as a sequential series of enzymic reactions is correct, a number of consequences may be predicted which can be tested experimentally. If a given gene affects only one enzyme and therefore only one biochemical reaction, each enzymic step should be controlled by a different gene. Or conversely, two non-allelic genes controlling even the same synthesis must affect different biochemical reactions in that synthesis. It should also follow that an intermediate compound preceding a genetically blocked reaction should be inactive, while one following this point in the biosynthesis might be expected to show the same order of activity as the end product.

The results of investigations have so far supported these predictions. At least seven different genes are known to be involved in the synthesis of arginine. (Srb and Horowitz, '44). Of these, four are concerned in unknown reactions leading to the synthesis of ornithine, two in the conversion of ornithine to citrulline, in which two biochemical steps have been suggested (Krebs, '36), and only one in the conversion of citrulline to arginine, a reaction involving only one obvious step. At least two genes are concerned in the synthesis of tryptophane, one in the production of anthranilic acid and one in the conversion of anthranilic acid

(125)